REMARKS

Applicants respectfully request reconsideration of the present application in view of the foregoing amendments and the following commentary.

I. Status of the Claims

Claims 1-30 were cancelled previously. Claims 31-47 are pending. No claim amendments are introduced by this response.

II. Rejection of Claims under 35 U.S.C. §103(a)

The Examiner rejected claims 31-47 for alleged obviousness over Ward *et al., Journal of Bacteriology* 182: 3239-3246, 2000 ("Ward"), in view of Jensen & Hammer, *Biotechnol. Bioeng.* 58: 191-195, 1998 ("Jensen1"), further in view of Jensen *et al., PNAS* 90: 8068-8072, 1993 ("Jensen2"), and further in view of de Vos, *Antonie van Leeuwenhoek* 70: 223-242, 1996 ("de Vos"). Applicants respectfully traverse the rejection.

The Examiner contends that the definitions for the phrases "glycolytic flux" and "reduced glycolytic flux" are "not explicit" due to the use of the phrasing "relates to" in the specification (Office Action, page 3, first full paragraph). Applicants respectfully disagree. One skilled in the art would have recognized, based on the teaching of the present invention, that the "reduced glycolytic flux" means the reduction of glycolytic flux in the claimed cells relative to the cells cultivated under aerobic conditions in the presence of a porphyrin compound (introduction of the respiratory metabolism) and in excess amount of lactose or glucose.

The present invention relates to a culture of lactic acid bacterial cells that are characterized by a reduced glycolytic flux and, under aerobic conditions, a respiratory metabolism, such that the culture displays a yield of biomass exceeding that obtainable from substrate-level phosphorylation. The reduced glycolytic flux is provided by introducing mutations in the cells to generate a lower rate of metabolism of the carbon source and the

respiratory metabolism is provided by introducing manipulations to the cells to produce an increased yield of ATP in the cells via oxidative phosphorylation when the cells are propagated in the presence of a terminal electron acceptor, as recited in claim 42.

Jensen2 describes the introduction of mutants into the chromosomal *atp* operon of *E. coli* and when the H^+-ATPase was over-expressed, the rate of glucose consumption gradually decreased in comparison to the wild-type cells. The decrease in glucose consumption is caused by natural regulation of the cells to maintain a desired level of ATP in the cells and to avoid excess ATP in the cells. The effect of increased H^+-ATPase on the intracellular ATP/ADP ratio is positive.

In contrast, the desired effect of the claimed invention is to reach a reduced ATP/ADP ratio. *See* specification, the paragraph bridging pages 13 and 14. Thus, Jensen2 does not disclose introducing manipulations to lactic acid bacterial cells to provide a reduced glycolytic flux since the uptake of glucose is not limited. In particular, Jensen2 does not suggest or motivate one skilled in the art to provide lactic acid bacterial cells, which are modified to produce a reduced glycolytic flux in combination with a respiratory metabolism.

Jensen1 relates to metabolic engineering strategies that allow modulations of gene expression in *Lactococcus lactis*. Jensen1 discloses that "the usual strategy [that] has been employed when attempting to ***increase the flux*** through a metabolic pathway..." (page 191, left column, lines 6-8 under "Metabolic Engineering and Metabolic Optimization," emphasis added), that "a second tempting strategy to use for ***increasing fluxes*** is to mutate/delete genes encoding enzymes involved in the production of 'undesired' products" (page 191, right column, lines 29-31, emphasis added), and that "...the optimization of that second enzyme has led to a ***significant increase in flux***" (page 192, left column, lines 9-11, emphasis added). Thus, similar to Jensen1, Jensen2 describes an increased glycolytic flux. Moreover, Jensen2 does not suggest introducing a respiratory metabolism into the lactic acid bacterial cells, alone or in combination with a reduced glycolytic flux.

Ward teaches cultivating *Enterococcus faecalis* in the presence of branched-chain α-keto acids for studying the regulation and the physiological role of the pathway provided by the *bkd* cluster. It is demonstrated that the branched-chain α-keto acids are converted to the corresponding free acids and resulting the formation of ATP via substrate level phosphorylation. Furthermore, it was demonstrated that utilization of branched-chain α-keto acids resulted in significant increase in biomass yield of 0.5 mole of ATP per mole α-keto acids metabolized. Nevertheless, Ward fails to teach a reduced glycolytic flux or lactic acid bacteria having mutations to generate a lower rate of the carbon source, let alone a lower rate of the carbon source in combination with specific manipulations to the cells that produce an increased yield of ATP in the cells via oxidative phosphorylation, as recited in claim 42.

As discussed in the response filed on September 12, 2007, de Vos teaches that the increase in the yield of biomass is obtained by increasing the activity of the enzymes involved in the uptake or degradation of a carbon source. As such, de Vos teaches an increased glycolytic flux rather than a reduced glycolytic flux as in the claimed invention. Moreover, de Vos does not suggest any lactic acid bacteria cells having a respiratory metabolism.

In view of the foregoing, the cited references, either alone or in combination, fail to suggest the claimed lactic acid bacterial cells that have a reduced glycolytic flux and a respiratory metabolism, which result in an increased yield of biomass. Accordingly, Applicants respectfully request withdrawal of the rejection.

CONCLUSION

Applicants believe that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested. The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check or credit card payment form being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicants hereby petition for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

Date May 28, 2008

By _____

FOLEY & LARDNER LLP
Customer Number: 22428
Telephone: (202) 672-5404
Facsimile: (202) 672-5399

Stephen A. Bent
Attorney for Applicant
Registration No. 29,768

by Yang Tang
Registration No. 55,663